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Step-Up and Step-Down Photoresponses in *Blepharisma*

Tatsuomi Matsuoka and Koji Taneda

Department of Biology, Faculty of Science, Kochi University, Kochi 780, Japan

ABSTRACT—The cells of *Blepharisma* responded to a step-up in light intensity (step-up response) by ciliary reversal, while the cells showed a temporal repression of ciliary reversal which was accompanied by swimming acceleration when light intensity was suddenly decreased (step-down response). The step-up response occurred only in anterior fragments obtained by bisection of the cells, while the step-down response occurred in both of fragments. The topographical difference in photosensitivity indicates that the photoreceptor systems responsible for the step-up photophobic response might be different from those for the step-down photoresponse (repression of ciliary reversal accompanied by acceleration of swimming velocity).

INTRODUCTION

The cells of *Blepharisma* show photodispersal that is caused by step-up photophobic response and changes in swimming velocity (photokinetic response) which depends on absolute light intensities [1]. The distribution of photoreceptor systems inducing the step-up photophobic response is different from that responsible for the photokinetic response; the photoreceptor systems responsible for the step-up photophobic response localize on anterior portion of a cell, while those for photokinetic response occur over the entire cell [2].

In the present study, we found that the cells of *Blepharisma* showed a repression of ciliary reversal accompanied by swimming acceleration in response to a step-down in light intensity. The present paper reports on the distribution of photoreceptor systems that cause these response.

MATERIALS AND METHODS

Blepharisma japonicum was cultured in 100-fold diluted lettuce juice at 23°C. The cells were collected by low-speed centrifugation, and washed in a standard saline solution containing 1 mM $CaCl_2$, 1 mM KCl and 5 mM Tris-HCl buffer (pH 7.2). Bisection of the cell was performed with a fine glass needle.

Prior to analysis of response, the motility of cells was recorded on a video tape through a dissecting microscope. White light intensity was varied by using neutral density filters, and was determined by a silicon photodiode (S1226-5BK, Hamamatsu Photonics Co.) photometer. To eliminate effects of heat rays, infrared-absorbing filters were placed in front of a light source. Temperature-controlled water (23°C) was circulated beneath the chamber to keep the temperature of the cell suspension

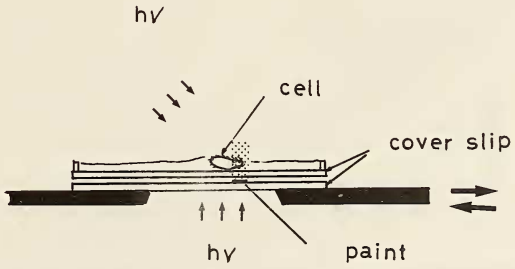

Fig. 1. A side-view of an apparatus for local photostimulation. A small circular region (diameter; 0.5 mm) of lower cover slip was painted black. The lower slip was fixed to a stage of microscope which could be manipulated. Upper cover slip (bottom of a chamber) on which the cell suspension was placed was fixed not to move. Double rays of white light (1.3 W/m^2 each) was applied to the cells for adaptation.

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constant, except for an experiment of local stimulation (Figs. 1, 3). To locally shade a cell, an apparatus shown in Fig. 1 was employed. Double beams of white light (1.3 W/m^2 each) were applied for at least 30 sec to adapt the cells. One of the beams was applied from the bottom, the other from the upper direction at an angle of 45°C against a horizontal line. In order to shade a certain portion of a cell, one of beams was obstracted with a small black-painted area (diameter; 0.5 mm) of a cover slip (Fig. 1, lower slip) which was placed just beneath a fixed chamber. This slip was fixed to a stage which could be manipulated in two dimensional directions.

RESULTS AND DISCUSSION

When light intensity was suddenly raised, most of intact cells of *Blepharisma* responded by ciliary reversal which continued for 15–20 sec (Fig. 2A), followed by a continuous increase in swimming velocity (Fig. 2B). On the other hand, the intact cells displayed a temporal repression of ciliary reversal (Figs. 2A, 2D) accompanied by a temporal increase in swimming velocity (Figs. 2B, 2E) when light intensity was suddenly decreased. The rate of the response gradually decreased to a constant level. anterior fragments responded by ciliary reversal in response to a step-up in light intensity, whereas posterior fragments did not (Fig. 2A). In intact cells, all of the cilia (even in posterior portion) can reverse in response to light irradiation. Therefore, the fact that the posterior

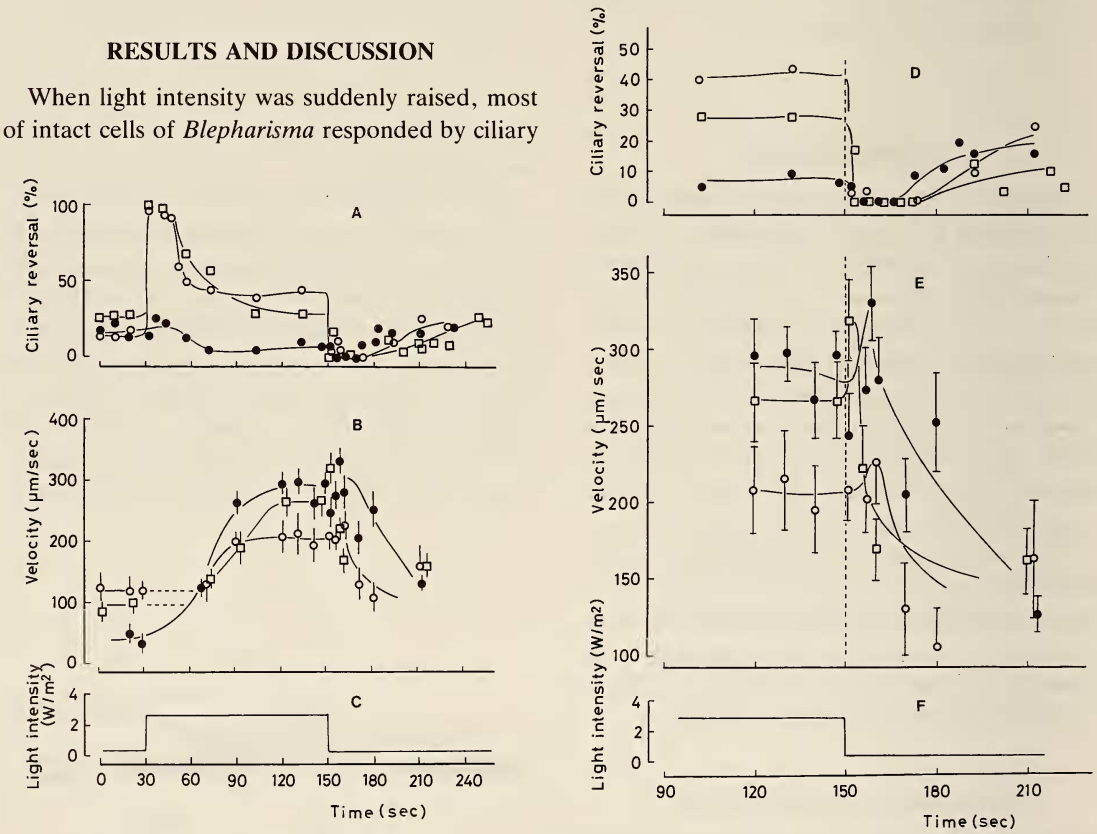

FIG. 2. Photoresponses of anterior fragments (open circles), posterior fragments (closed circles) and intact cells (open squares) of *Blepharisma* to a step-up and step-down in light intensity. (A) Changes of degree of ciliary reversal to stepwise changes in light intensity. The degree of the response was expressed as the percentage of the total number (n=30–40 cells) showing ciliary reversal in 5 sec. (B) Changes of forward swimming velocity to stepwise changes in light intensity. Bars correspond to the means and SE (determinations of 25–30 cells). (C) Changes in light intensity with time (sec). Light intensities were changed between 0.27 and 2.7 W/m^2. The swimming velocity was not measured in a period (dashed line) in which cells rerponded by ciliary reversal. The swimming velocity was determined by tracing the forward swimming cells for 0.5 sec. (D), (E) Scaled-up figures of the step-down responses showed in (A) and (B). (F) Changes in light intensity with time (sec).

fragments can not respond indicates that photoreceptor systems for the step-up photophobic response do not exist in the posterior portion. However, strong light irradiation of the posterior fragments often induced a slight increase in degree of ciliary reversal. This may be attributed to existence of a few photoreceptor systems around middle portion of a cell where the cell is bisected. When light intensity was decreased, both of the fragments responded by a temporal repression of ciliary reversal (Figs. 2A, 2D) and swimming acceleration (Figs. 2B, 2E). In bisected fragments, swimming acceleration occurred in 4–5 sec after light intensity was decreased, although the intact cells responded just after stimulation (Fig. 2E). The delay of the response in bisected fragments may be due to some damages by bisection.

Uusing intact cells, a local stimulus (a step-down in light intensity) was applied. When either the anterior or posterior was shaded, swimming velocity of the cells increased (Fig. 3). The results also suggest that the photoreceptor systems inducing the step-down response distribute over the entire cell. Degree of temporal acceleration of swimming velocity of the cells which were locally shaded (Fig. 3) was different from that of the cells entirely shaded (Fig. 2). This may be attributed to reasons as follows: (1) Differences in excitability of different cell populations. (2) Temperature effect on the response by light irradiation (In Fig. 3, we failed to control the temperature of cell suspension, because gap between two sheets of cover slips reduced the conduction of heat).

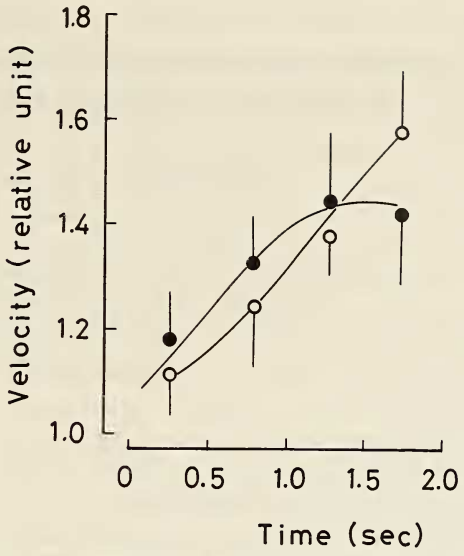

FIG. 3. Acceleration of forward swimming velocity of the intact cells of *Blepharisma* which were locally shaded. The forward swimming velocity (relative unit) was expressed as the rate of the velocity of the cell in 1 sec before the step-down stimulation was applied. Before shading, the cells were adapted to the double beams of white light at least 30 sec. Open and closed circles indicate the forward swimming velocities of the cells shaded in anterior and posterior portions, respectively. Points and bars correspond to the means and SE (determinations of 20–30 cells). The velocity was determined by tracing the swimming cells for 0.5 sec. The cells were shaded in about 1/3 portions from the anterior or posterior end. Duration of the shading was 0.2–0.5 sec. Abscissa; time course (sec) after cells were locally shaded.

The topographical difference in photosensitivies of the cells of *Blepharisma* to a step-up and step-down in light intensity indicates that photoreceptor systems mediating these responses show in different distribution. In *Blepharisma,* ciliary reversal is caused by an increase in intracellular Ca^{2+} concentration [3] as well as *Paramecium* [4]. In *Paramecium bursaria,* depolarizing photoreceptor potential which is probably mediated by localized photoreceptor systems [5] is caused by a transient increase in the membrane conductance to Ca^{2+} [6]. It is presumed that step-up photophobic response (ciliary reversal caused by the step-up in light intensity) of the cells of *Blepharisma* may be mediated by an activation of photoreceptor Ca channels. Localization of photosensitivity of the cells of *Blepharisma* to the step-up in light intensity is possibly attributed to that of photoreceptor Ca channels associated with photoreceptor pigments. In *Paramecium,* mechanical stimulation of the anterior portion induces a trasient depolarization of the membrane triggered by an activation of mecahnoreceptor Ca channels that is responsible for ciliary reversal, whereas stimulation of the posterior portion leads a temporal hyperpolarization of the membrane triggered by an activation of mechanoreceptor K channels which might be involved in a temporal acceleration of swimming velocity [4, 7, 8]. In *Blepharisma,* a temporal acceleration of swimming velocity induced by a

step-down in light intensity might be responsible for photoreceptor K channels as well as the mechanoreceptor K channels in *Paramecium*. If so, distribution of photosensitivity of the *Blepharisma* cells to the step-down in light intensity may be due to the distribution of photoreceptor K channels associated with some photoreceptor pigments.

The acceleration of swimming velocity induced by a step-down in light intensity is a temporal photoresponse. In contrast, an increase in swimming velocity of *Blepharisma* caused by a step-up in light intensity, which is not temporal response, is possibly related to continuous hyperpolarized membrane potential, because continuous irradiation of strong light reduce frequency of spontaneous ciliary reversal (unpublished).

Preparation works showed that action spectrum for the step-up photophobic response (ciliary reversal) had three peaks at 580, 540 and 480 nm in visible range. Further examinations involve, by determining action spectrum for the swimming acceleration caused by a step-down in light intensity, to compare the action spectrum for the step-up photophobic response with that for the step-down swimming response.

Photoreceptor systems responsible for the step-up photophobic response (ciliary reversal) localize anterior portion of the cell; especially anterior end of the cell is most sensitive to light [2]. The cells of *Blepharisma* avoid from light by step-up photophobic response [1]. Therefore, such a localization of photoreceptor systems might be significant, because anterior portion of cell faces light source when the cell is swimming toward light source. On the other hand, swimming acceleration induced by a step-down in light intensity might contribute for the cell to rapidly escape for light when the cell is swimming toward darker region. Hence, photoreceptor systems responsible or the step-down response (swimming acceleration) would distribute over the entire cell.

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